Validating Pathway Analysis of Organic Contaminants from Aged Dredged Material Using Plants and Worms

PURPOSE: Contaminants in dredged material (DM) placed in an upland situation, such as a confined disposal facility (CDF), may move from substrates into food webs because of their contact with CDF-colonizing or –inhabiting plants and animals, and therefore may cause unacceptable risks outside the CDF. The primary goal of this technical note is to provide guidance on evaluating bioaccumulation of organic contaminants and toxicological effects in test species representing two trophic levels of a food web.

BACKGROUND: Placement of DM in CDFs and its removal from CDFs for beneficial use require assessment of environmental risk. To this end the decision-making framework and the U.S. Army Corps of Engineers (USACE)/U.S. Environmental Protection Agency (USEPA) Technical Framework may require exposure and effects assessments for plants and animals of relevant contaminant pathways prior to dredging. Currently no specific guidelines for contaminant residues in plants and animals exist. The purpose of this study, sponsored by the Dredging Operations and Environmental Research (DOER) Program, was to develop and validate methods or further validate existing methods to assess the potential for unacceptable environmental risk outside a CDF filled with contaminated DM, and to assess the suitability of aged materials for beneficial use outside the CDF.

INTRODUCTION: USACE annually manages about 300 million cubic meters of DM. Historically about 50 percent is disposed of in open water, 40 percent is used for beneficial purposes and the 5 to 10 percent of the material that is contaminated and unsuitable for open-water disposal is placed into CDFs. USACE Districts require tools to help integrate topical quantitative information to yield quantifiable estimates of risk posed by dredged materials including uncertainty (Moore et al. 1998). The use of effects-based testing and risk assessment is intended to supplement the analytical options currently available to DM managers by building on the existing technical framework (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers (USEPA/USACE) 1992) and the existing tiered approaches (USEPA/USACE 1991, 1998; USACE 2003).

Test Organisms Used for Contaminants in Dredged Material: Several test organisms have been used by USACE to evaluate bioaccumulation of metals from DM disposed in an upland situation (Folsom et al. 1981, Van Driel et al. 1985, Folsom and Price 1989, Simmers et al. 1989). Unfortunately, in several of these studies the toxic effects of metals were sometimes noted but not quantified, nor were tests for toxic effects of organics performed. In a recent study, *Cynodon dactylon* (bermudagrass) and *Eisenia fetida* (earthworm) were used as test organisms for metals in DM from freshwater origin (Best et al. 2003). Bermudagrass was relatively sensitive to zinc, which was the metal of concern in the test material, and could, therefore, only serve for bioaccumulation and toxicity assessment at lower Zn levels, e.g. for beneficial use of DM.

The use of this grass species is limited to the southern part of North America as far north as Kentucky. Earthworms did not exhibit metal toxicity in the range studied, and a correlation was found between bioaccumulation and substrate Zn levels. Thus, earthworm bioaccumulation was considered as a valid Tier III assay for Zn (Best et al. 2003).

Behavior of Organic Contaminants in Soils, and Consequences for Ecotoxicity Tests: Organic compounds differ from metals with respect to their behavior in the soil (e.g. organic contaminants are biodegradable, while metals are not) and toxic action. Their interaction with plants differs from that of metals. Moreover, ecotoxicological research on organic contaminants is far scarcer than on metals. The bioavailability of organic contaminants can change significantly over time due to complexation, degradation, or loss by volatilization, runoff, or leaching.

Hydrophobic organic contaminants (HOC) in sediments/soils and dredged material can exist in any of the following four states: solids (soil and organic matter particles), fluid (water), gas (within the sediment/soil), and biota (Brusseau 1997). Partitioning of the contaminant over these phases depends on the contaminant characteristics and the processes affecting the transitions from one phase to the other. The most important phase transition processes are the solubilization (transition of pure contaminant into the water phase), volatilization (transition from the water to the gas phase), and sorption (binding to the solid phase; Beck et al. 1993). The most immobile HOCs are hydrophobic (low solubility in water) and lipophilic. These contaminants are usually strongly bound to organic material and sediment/soil particles (Tenner et al. 1997). These characteristics decrease bioavailability and toxicity for plants (Shimp et al. 1993), soil macrofauna (Belfroid et al. 1995), surface fauna, and microorganisms. Bioavailability of HOC decreases with aging (Alexander 1995). Therefore, short-term laboratory experiments in which spiked substrates are tested may lead to overestimates of the ecotoxicological risks of aged contaminants. The aging process results in adsorption of HOC in micro- or nanopores and/or the soil matrix. This means that strongly bound, non-extractable, or difficult to extract HOC residues can be present in the soil long after HOC pollution took place. Although the concentrations of aged HOCs may be high, ranging from 100 to 1000 mg/kg organic soil matter, desorption processes are extremely slow, and, therefore, the relationship between total HOC concentration and ecological processes in the same soil is difficult to establish (De Jonge 1996).

The organic carbon fraction of DM can play an important role in the bioavailability of HOCs (Fredrickson et al. 2003). A large fraction of the HOC that is extractable from sediments with organic solvents can be non-available to biota, and the proportion of the non-biologically available fraction to total extractable HOC levels can vary greatly from sediment to sediment. For instance, Talley et al. (2002) showed that Soxhlet-extractable PAH concentrations in DM from a CDF in Milwaukee Harbor averaged 115 mg kg^{-1}, but only 46 mg kg^{-1}, i.e. less than half, was bioavailable. These results and others have led to questioning the validity of the equilibrium partitioning theory developed by DiToro et al. (1991), that describes bioaccumulation of HOCs from sediments by a simple constant partitioning process between sediment and organic carbon and biomass (Kraaij et al. 2002). The quality of organic carbon may also have a large effect on the bioavailability of HOCs. For instance, in several studies the non-bioavailable fraction of solvent-extractable HOCs was associated with pitch and coke-derived particles (Paine et al. 1996; Talley et al. 2002).

Four exposure pathways for HOCs are distinguished in plants (Wild et al. 1992): (1) passive root uptake (with the transpiration flux) from the soil solution, (2) leaf uptake of compounds evaporated from the soil surface, (3) direct contact between roots and/or leaves with contaminated soil particles, and (4) active uptake through oil channel systems in the roots. The first two routes are common, the last two routes pertain to specific conditions and/or plant species (Ryan et al. 1988). Uptake route 1 is relevant for HOCs with a low volatility, uptake route 2 for HOCs with a high volatility (Ryan et al. 1988). Passive root uptake from the soil solution is similar to the partitioning of the compound between water and solid phase. Root uptake is usually related to the measure for lipophily, the octanol/water partitioning coefficient (log K_{OW}). Root-to-shoot translocation is generally related to the log K_{OW} as well as the evapotranspiration rate. Translocation of HOCs with a low log K_{OW} (<4) is likely. HOCs with a log $K_{OW} \geq 4$ are well-adsorbed to soil particles, organic matter and plant roots, and are not very mobile in the soil solution. The total root uptake depends on the average concentration of the HOC during the whole exposure period of the plant up to harvesting. Based on log K_{OW} characteristics of various HOCs, Ryan et al. (1988) conclude that PAHs (e.g. benzoapyrene, log K_{OW} of 6.4) and PCBs (e.g. Arochlor 1254, log K_{OW} of 6.0) will bind strongly to the root surfaces, but may not be transported from roots to shoots. No evidence currently exists on the relative sensitivity of mono- versus dicotyledonous plants for HOCs (Fletcher et al. 1987, Hund and Traunspurger 1994).

Earthworms are suitable bioaccumulation and response indicators for metals as well as for HOCs (ASTM 1998, Kula and Larink 1998, Lokke and Van Gestel 1998). Toxicological effects in this test organism originate largely from direct skin contact with the toxic compounds in the interstitial water. Chronic sublethal tests are preferred above acute toxicity tests since the former generate information not only on toxicity effects on biomass but also on body burdens. These tests currently exist (Doube and Schmidt 1997, Van Gestel 1997).

Frequently used measures to describe toxicity are: the lethal concentration (LC), i.e. the concentration of a toxicant that kills a specified percentage of the organism; the effective concentration (EC), i.e. the concentration of a toxicant that produces an observable negative effect in the organism; and the phytotoxicity threshold (PT), i.e. the contaminant tissue concentration of a plant that corresponds with a defined growth reduction (ASTM 1994; ASTM 1998). The importance of phytotoxicity data for estimates of the risk posed by the soil contamination is evident (Benenati 1990), but plant data are currently not widely used in setting policy for soil or sediment cleanup limits. For the beneficial use of dredged material, phytotoxicity should be minimized since it inhibits the biomass production of vegetation on the soil.

Test Organisms Selected for Organic Contaminants in Dredged Material: The monocotyledonous *L. perenne* (perennial ryegrass) was selected for its worldwide use and general acceptance in standard test procedures (USEPA 1996; USEPA 1999). It has a wide geographical distribution, rapid growth, and profuse generative reproduction. In addition, its seeds germinate simultaneously within several days and the species can be cultivated in the testing environment. *L. perenne* is relatively tolerant towards HOCs, and is used widely as a response and bioaccumulating indicator for HOC contamination of soils (Gorsuch et al. 1990; Van de Leemkuile et al. 1998; Malmberg et al. 1998). A related species, *Lolium multiflorum* (ryegrass) is widely used in Germany as a bioaccumulating indicator for organic contaminants (*http://www.stadtentwicklung.berlin.de/umwelt/monitoring/en/wirkungskadaster/*). *C. esculentus*

(yellow nutsedge), frequently used for DM testing, was not used for the current tests, because attempts to enhance and synchronize germination of its tubers were unsuccessful.

The earthworm *E. fetida* was selected for its worldwide use and acceptance, facilitating comparison with bioaccumulation and toxicity data of other sites, and ease of culture under laboratory conditions. *E. fetida* and *E. andrei*, the most used laboratory test species, exhibit a litter-dwelling ecological strategy, live in organic matter-rich soil, and reproduce via cocoons. Toxicological effects in these test organisms originate largely from direct skin contact with the toxic compounds in the interstitial water. Earthworms are suitable bioaccumulation and response indicators for metals as well as HOCs (ASTM 1998; Kula and Larink 1998; Lokke and Van Gestel 1998). Jager (1998) discusses earthworm ecological strategies and models to estimate the bioconcentration of organic chemicals by earthworms.

Study Objectives: The current study is aimed at identifying/validating suitable herbaceous plant and worm species as test organisms for organic contaminants in DM from freshwater origin. The specific objectives were to evaluate in terrestrial plants and worms:

(1) Bioaccumulation of organic contaminants from organics-contaminated DM.

(2) Chronic toxicity, as indicated by decreasing biomass on organics-contaminated DM.

MATERIAL AND METHODS

Bioaccumulation and Toxicity Assays. A dilution series was constructed by mixing Indiana Harbor Canal (IHC) DM with reference DM. Subsequently, dose-response curves for organics concentrations between 0 and 70 percent IHC (46 mg Arochlor-1248 kg^{-1} dry weight (DW) and 336 mg PAH kg^{-1} DW) were constructed for both plant and animal tests. Plant and animal tests were replicated five times, and followed a randomized block design. Desorbability of the organic contaminants, as a measure for potential bioavailability, was evaluated using C18 disks, replicated three times. No factors other than percent IHC-DM contribution to the test substrate were tested because only a limited amount of IHC-DM was available. DM with concentrations of <1 mg PCB kg^{-1} DW (Monroe CDF, MI; Best et al. (2003)) and 115 mg PAH kg^{-1} DW may be considered representative for DM from harbors similar to IHC (Milwaukee Harbor, Talley et al. 2002).

The following responses were measured:

- For plants:
  - Accumulation, as measured by the plant tissue-organics concentrations accumulated in 59 days, in mg kg^{-1} DW and in mg kg^{-1} organic carbon (OC).
  - Toxicity, as measured by the plant biomass formed in 59 days, in g DW m^{-2}.
- For worms:
  - Accumulation, as measured by the worm tissue-organics concentrations accumulated in 28 days, in mg kg^{-1} DW and in mg kg^{-1} OC.
  - Toxicity, as measured by the biomass of 12 worms after 28 days incubation, in g DW $cylinder^{-1}$.

- For C18 disks:
  - o Sorption, as measured by the disk-organics concentrations accumulated in 28 days, in mg kg^{-1} DW.

Substrates. Two dredged materials (DM) were used in the tests. The contaminated DM from the field site was chosen because of the interest of the U.S. Army Corps of Engineers. The latter DM was obtained from an area planned for dredging and disposal in CDFs. It was collected in 1991 from the Indiana Harbor Canal (IHC), IN (Figure 1), and stored in a 55-gal drum in a wet state at room temperature until use. The IHC DM contained significant amounts of polychlorinated biphenyls (PCBs, i.e. Arochlor 1248) and polyaromatic hydrocarbons (PAHs), but also high levels of the metals cadmium (Cd), chromium (Cr), nickel (Ni) and zinc (Zn). The reference DM originated from the nearby Monroe, MI, CDF, where it was excavated from the substrate surface to a depth of 0.33 m at a dry site, in June 2002. DM from the same site was used as a reference in two earlier bioassays (Best et al. 2001; 2003). Properties of both DMs are listed in Table 1. Both DMs were dried to a moisture content of approximately 5 percent, and ground to pass a 2-mm sieve. During this process, part of the volatile PAHs was lost from the IHC DM, amounting to 96 percent for naphthalene and 70 percent for acenaphthene and fluorene.

The substrate dilution series was constructed by mixing different amounts of IHC DM with reference DM (Table 1). A range of four dilutions up to approximately 70 percent IHC DM was created, containing 46 mg kg^{-1} DW Arochlor-1248 and 336 mg kg^{-1} DW PAH. Dilutions were used because it was expected that the high metal concentrations in the IHC DM would cause acute toxicity in the test organisms, and thus prevent determination of bioaccumulation of organic contaminants in the plants and worms.

As a control substrate for plants, Baccto R Lite potting soil, Michigan Peat Company, Houston, TX, was used. As a control substrate for worms, a standard artificial soil (Organization for Economic Cooperation and Development (OECD), Paris, France; Kula and Larink 1998) was used.

Plant Tests. The plant tests used the following treatments:

- **Percent IHC DM:** Five levels:
  - o Reference (0 mg A-1248, and 0.32 mg PAH kg^{-1} DW)
  - o 10 percent (10 mg A-1248, and 66.1 mg PAH kg^{-1} DW)
  - o 20 percent (17 mg A-1248, and 114.7 mg PAH kg^{-1} DW)
  - o 33 percent (24 mg A-1248, and 183.2 mg PAH kg^{-1} DW)
  - o 70 percent (46 mg A-1248, and 336.3 mg PAH kg^{-1} DW)
- **Moisture:** Two levels:
  - o Approximately 1/3 field capacity (15 percent)
  - o Close to field capacity (36 percent, field capacity being 38 percent). A moisture level at field capacity allows maximum mobility of contaminants in soil solution.
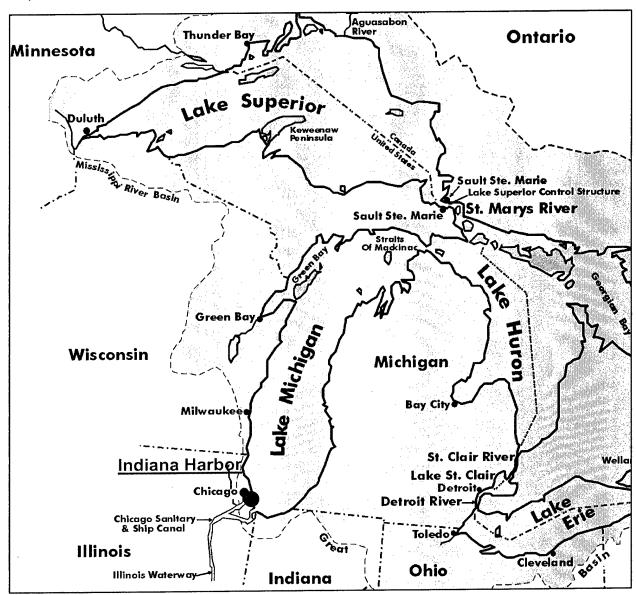
Figure 1. Indiana Harbor Canal, IN, from which the dredged material for the tests originated

A control substrate served as a test to verify performance of the plants; five replicates at a 36-percent moisture level were tested. The plant study included a total of 55 units. For each unit, 0.230 g *L. perenne* seeds were weighed and placed on top of 1 L of the appropriate substrate mixture contained in 2-L plastic pots. Plants were amended with slow-release Osmocote fertilizer after 10 days to attain target levels of 352 kg N ha^{-1}, 59.2 kg P ha^{-1} and 331.9 kg K ha^{-1}, commonly used for pastures (Best and Jacobs 2001). Plants were allowed to grow for 59 days prior to harvesting. Seeds germinated synchronously, as was verified before the onset of the tests. *C. esculentus*, initially considered as another suitable candidate for a plant test species, was not used for the current tests, because attempts to enhance and synchronize germination of its tubers were unsuccessful.

Animal Tests. Animal tests used the same treatments as the plant tests, but were carried out at only one moisture level, 36 percent. The invertebrate study included a total of 30 units. For each

unit, 12 *E. fetida* specimens were placed on top of 1 L of the appropriate substrate mixture contained in a 15-cm-diameter, 15-cm-high Plexiglass cylinder. Animals were allowed to grow for 28 days prior to harvesting (ASTM 1998).

C18 Disk Tests. The C18 disk test was conducted to assess the potential bioavailability (desorbability) of the organic contaminants in the substrates. The C18 disk study included a total of 15 units. For each unit, one preconditioned C18 disk (SPE disk ENVI 18 DSK, diameter 47 mm, Supelco, Burdick and Jackson, Muskegon, Michigan) was suspended in a 250-mL Erlenmeyer containing 10 g DW test substrate and 50 mL Reversed Osmosis (RO) water. One mL of a solution containing 7 mmol of $HgCl^2$ L^{-1} was added to each bottle to reduce microbial activity. Bottles were incubated for 28 days in the dark at 22 °C and shaken gently every second day prior to analysis. The organics reached near-equilibrium on the disks in 28 days, as was verified before the onset of the tests (Krauss and Wilcke 2001, Krauss et al. 2000).

Analyses. Prior to incubation, each DM mixture was analyzed for chemical and physical characteristics, in triplicate. The concentrations of PCBs and PAHs were determined in all DM mixtures, following a modified Bligh-Dyer extraction procedure (Bligh and Dyer 1959) and Gas Chromatography/Mass Spectrometry detection (GC-MS; EPA-method 8270C). For Arochlor-1248, the PCBs with molecular weights of 222, 256, 292, and 326 were quantitated as representatives. No other Arochlors were present in the IHC extracts. For the PAHs, the following constituents were quantitated:
- Naphthalene (NAP)
- Acenaphthalene (ACY)
- Acenaphthene (ACE)
- Fluorene (FLU)
- Phenanthrene (PHE)
- Anthracene (ANT)
- Fluoranthene (FLA)
- Pyrene (PYR)
- Benz[a]anthracene (BAA)
- Chrysene (CHR)
- Benzo(b)fluoranthene (BBF)
- Benzo(k)fluoranthene (BKF)
- Benzo(a)pyrene (BAP)
- Benzo(ghi)perylene (BGHI)
- Indeno(1,2,3-cd)pyrene (INP)
- Dibenzo(a,h)anthracene (DIB)

As internal standard for the PCB extraction, PCB No. 7 was used, which was spiked to the DM samples prior to extraction. The total concentrations of As, Cd, Cr, Cu, Pb, Ni, V, and Zn were also determined (USEPA 1991), as were the bioavailable metal concentrations, using the diethyltriamine-pentaacetic (DTPA) extractable fraction as a measure (Lindsay and Norvell 1978). Moisture content was determined by drying at 105 °C in a forced-air oven until constant weight. Concentrations of organic matter were determined by loss on ignition at 550 °C, and bulk density volumetrically (Allen et al. 1974). Organic carbon in the substrates was calculated by multiplying the organic matter concentration by a factor of 0.58 (Jackson 1964). pH_{KCl} was measured with a pH-meter (Beckman Model PHI40, Fullerton, CA) in a 1-M KCl solution in a

fresh soil-to-liquid ratio of 1:2.5 (w/v). pH_{KCl} was converted to pH_{water} using a regression equation of $pH_{water} = 0.677 \times pH_{KCl} + 2.35$ (ISO 10390; Best and Jacobs 2001).

Dry weight (plants and worms) was determined by drying of the fresh material in a forced-air oven to constant weight (105 °C). PCB and PAH concentrations were determined in the fresh materials of three of the five replicate plant and animal samples, using a similar approach as for the DM mixtures. Lipid contents were determined gravimetrically in subsamples of the plant and worm dichloromethane (DCM; Bligh and Dyer 1959) extracts. Total metal concentrations were determined in the remaining two replicate plant and animal samples, including As, Cd, Cr, Cu, Pb, Ni, and Zn, using an approach similar to that used for the DM mixtures, but digesting 0.3 g DW. These latter determinations in plants and worms allowed exploration of factors contributing to the untimely demise of the test organisms.

PCB and PAH concentrations were determined in hexane extracts of all C18 disks (Krauss and Wilcke 2001). Recoveries of the internal standard were on average 40 percent in DM mixtures, 48 percent in DM mixtures, 65 percent in plants, 81 percent in worms, and 48 percent in C18 disks.

Statistics. The STATGRAPHICS Plus for Windows 3 package (Manugistics, Rockville, MD; 1997) was used for statistical analyses.

Effects of treatment on plant and worm responses were tested for statistical significance using analysis of variance (ANOVA), followed by a multiple range test. The p-value in the ANOVA is a measure of the significance of the analysis; it was set at a 95-percent confidence level (p value ≤ 0.05).

Relationships between plant and worm responses and substrate contaminant concentrations were derived by linear regression. The p-value in the regression model was set at a 95-percent confidence level (p value of ≤ 0.05) unless stated otherwise. The R^2-value of the regression model indicates the proportion of the variance explained by the model; regressions with R^2-values of 0.50 explain 50 percent of the variance in the dataset.

RESULTS AND DISCUSSION

Substrates. The five DM mixtures spanned organics concentration ranges of 0 to 46 mg Arochlor1248 kg^{-1} DW for PCBs and of 0.32 to 336 mg PAH kg^{-1} DW (Table 2). The levels of total As, Cd, Cr, Cu, Pb, Ni, and Zn were far higher in the DM mixtures than in the reference DM, and the DTPA-extractable metal levels were far lower than the total metal levels. Based on the published critical stressor concentration levels (CSCL), largely identical with the "No Observed Adverse Effect Level" (NOAEL) or "Lowest Observed Adverse Effect Level" (LOAEL), it was expected that the concentrations of Cd and Zn even in the least diluted IHC DM mixture might be deleterious for plants as well as worms.

Bioaccumulation and Toxicity in Plants: In the plant material, Arochlor1248 was mainly recovered from the roots. Arochlor1248 concentrations in root tissues increased with those in the DM mixtures (Table 3). This led to the conclusion that Arochlor1248 was sorbed and/or taken up by the roots, but not translocated to the shoots. Arochlor1248 recovered from the plant shoots

cultivated on the 33-percent IHC DM mixture may have been adsorbed to the tiny roots anchoring the plants to the substrate. These roots were so small that they were not harvested separately. It may also have been adsorbed through volatilization from the substrate surfaces. Concentrations in plant roots from dry DM mixtures were far higher than from wet DM mixtures.

PAHs (sum of 16 PAHs) were recovered from roots and shoots (Table 3). PAH concentrations were higher in roots than in shoots and they increased with increasing PAH concentration in the DM mixture. This may indicate that most PAHs were taken up by the plants and translocated from roots to shoots, since a large part of the volatile PAHs had been lost during drying of the IHC-DM (Material and Methods Section 'Substrates' p. 5). The high PAH concentrations in plant shoots cultivated on the 33-percent IHC DM mixtures may also have been adsorbed to the tiny roots anchoring the plants to the substrate, as suggested for the PCBs. PAH concentrations were far higher in plant roots from dry than from wet DM mixtures. No significant relationships between organics concentrations in plant tissues and those in DM mixtures, both expressed as milligrams compound per kilograms dry weight, were found. However, upon further inspection of the data, significant relationships were identified; these are discussed under "Relationships between concentrations of individual organics in substrates and tests using plants, animals, and C18-disks," p. 10.

In the plant material, various metals accumulated up to considerable concentrations (Table 3). Shoot concentrations of Cr, Pb, and Zn exceeded CSCLs for plants in the dry 20-percent IHC mixtures, while the concentration of Zn came close to the phytotoxic level recently determined for another grass species, *Cynodon dactylon* (bermudagrass; Best et al. 2003).

Plant biomass was significantly affected by percent IHC contribution to the DM-mixture, as found by ANOVA ($p<0.05$; Table 3). Substrates with an IHC-DM contribution exceeding 33 percent did not support plant growth. The decrease in plant biomass with increasing IHC-DM percent was tentatively attributed to the various metals in the DM mixtures, but no single metal could be identified that explained this biomass decrease using multiple regression techniques. Biomass was significantly higher in plant material harvested from the wet than from the dry DM mixtures ($p<0.001$; Table 3). The biomass of plants exposed to DM mixtures, including the reference, was always lower than that of the control plants (226 ± 23.45 g DW m^{-2}), indicating that all DM mixtures provided less than optimal substrates for biomass formation.

Bioaccumulation and Toxicity in Worms: In the worms, both Arochlor1248 and PAHs accumulated. Arochlor1248 and PAH concentrations in worm tissues were significantly affected by percent IHC contribution to the DM mixtures, as found by ANOVA, with PAHs accumulating more gradually than PCBs (Table 4). No significant relationships between organics concentrations in worm tissues and those in DM-mixtures, both expressed as mg compound per kg dry weight, were found.

In the worms, various metals accumulated up to considerable concentrations (Table 4). In the DM mixtures containing ≥ 10 percent IHC-DM, only the zinc concentrations exceeded the CSCL for worms.

Worm biomass was significantly affected by percent IHC contribution to the DM-mixture, as found by ANOVA ($p<0.05$; Table 4). The decrease in worm biomass with increasing IHC-DM contribution was also in this case tentatively attributed to the concentrations of various metals in the DM-mixtures, but no single metal could be identified that explained this biomass decrease using multiple regression techniques. The biomass of worms exposed to DM mixtures containing >20 percent IHC-DM was lower than that of the control worms (1.74 ± 0.11 g DW cylinder^{-1}; 19.8 ± 0.23 individuals), but worm biomass was higher on the other DM mixtures than that of the control, indicating that the latter DM mixtures provided acceptable substrates for biomass formation.

Relationships Between Concentrations of Individual Organics in Substrates and Tests Using Plants, Animals, and C18 Disks

No significant relationships between total organics concentrations in plants and worms, and those in DM-mixtures, both expressed in mg compound per kg dry weight, were found.

However, statistically significant linear regressions were found between the ^{10}log values of selected, individual PCB and PAH compounds, expressed as mg compound per kg lipid of plants and animals, expressed as mg compound per kg disk and per kg soil-DW of C18 disks, and the ^{10}log values of the same compound in the DM-mixtures, expressed as mg compound per kg organic carbon (OC) (Table 5).

These relationships were statistically significant in:
- Plant shoots
  : at a 95-percent confidence level for FLU, FLA, PYR, and BAA.
  : at a 90-percent confidence level also for PHE and BBF.
- Plant roots
  : at a 90-percent confidence level for ANT and PYR.
- Worms
  : at a 95-percent confidence level for ACE, FLA, IND, BGHI, and DIB.
  : at a 90-percent confidence level also for BBF.
- C18-Disks
  : at a 95-percent confidence level for all organic compounds except NAP and DIB.

Based on the established significant linear regressions between the individual organic compounds in the DM mixtures and the test devices, the authors conclude that *L. perenne* shoots can be used as a bioaccumulation indicator for 6 of the 16 PAHs (i.e., FLU, PHE, FLA, PYR, BAA, and BBF); roots only for 2 of the 16 PAHs (i.e., ANT and PYR); and earthworms for 6 of the 16 PAHs (i.e., ACE, FLA, BBF, IND, BGHI, and DIB). The C18 disks represent a method suitable to quantify the potential bioavailability (desorbability) of most organics (i.e., 14 of the 16 PAHs) and of Arochlor-1248.

The transfer of the organic compounds from the DM mixtures into the plants and worms was far more irregular than into the C18 disks (Table 5). This was attributed in part to the lower mobility of the compounds in the consolidated DM to which the plants and worms were exposed (versus that in the slurries to which the disks were exposed) and in part to species- and trophic level-specific selectivity of the plants and worms for individual compounds. The latter suggestion is

supported by the fact that only one organic compound was identified (BBF) for which both plants and worms showed significant transfer from the DM mixtures.

CONCLUSIONS: Results of this study revealed the following:

- Perennial ryegrass responded to increasing levels of Arochlor-1248, PAHs, and various metal contaminants in DM mixtures by exhibiting increased bioaccumulation and toxicity.

- Substrates with an IHC-DM contribution exceeding 33 percent did not support plant growth. This may indicate that this plant species may be too sensitive to project organics bioaccumulation from DM, containing both organics and high levels of metals.

- Perennial ryegrass may be used for bioaccumulation of (selected) PAHs at lower metals levels, e.g. for beneficial use of DM, in North America.

- ^{10}Log-transformed PAH concentrations on a plant-lipid basis were accurate predictors of ^{10}log-transformed PAH concentrations on a DM-organic carbon basis for 6 of the 16 PAHs (i.e. FLU, PHE, FLA, PYR, BAA, and BBF), but not for PCBs.

- Earthworms also showed an increased bioaccumulation and decreasd biomass in response to increasing levels of Arochlor-1248, PAHs, and metals contamination. Because the worms survived on all DM mixtures, they may be considered as tolerant enough to project organics bioaccumulation from DM, containing both organics and high levels of metals.

- The earthworms were just as reliable in accumulating PAHs as the plants, but for different compounds.

- ^{10}Log-transformed PAH concentrations on a worm-lipid basis were accurate predictors of ^{10}log-transformed PAH concentrations on the DM-organic carbon basis for 6 of the 16 PAHs (i.e. ACE, FLA, BBF, IND, and BGHI), but not for PCBs.

- C18 disks were reliable devices to project potential bioavailability of almost all organics present; i.e. for 14 of the 16 PAHs (ACY, ACE, FLU, PHE, ANT, FLA, PYR, BAA, CHR, BBF, BKF, BAP, IND, BGHI, DIB) and for Arochlor-1248.

- Plant and earthworm bioaccumulation may be Tier III and valid Tier IV assay (Upland Testing Manual 2003), provided more work is done on the bioavailability and selectivity of metal-tolerant, test organisms for organics, and on the desorption and mobility of organics in sediments and soils. C18 disks certainly look promising.

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Table 1
Characteristics of Dredged Materials Used to Create the DM Mixtures for the Test and Control Soils[1]

	Dredged Material		Soil		USEPA Guideline		Eastern United States
Characteristic	Indiana Harbor Canal	Monroe-CDF dry	Plant Control	Invert. Control	CSCL plants	CSCL earthworms	
Organics (mg kg^{-1} DW)							
Arochlor-1248	44.20	0	0	0	40	-	-
Σ16-PAHs	389.0	0.32	0	0	-	500[2]	
Total Metals (mg kg^{-1} DW)							
Antimony	5.59	<2.68			5	-	33000
Arsenic	61.4	3.49			10	60	4.8
Cadmium	15.23	0.65	1.24	<2.0	4	20	--
Chromium	569.3	16.93			1	0.4(VI)	33
Copper	293	19.77			100	60	13
Lead	763	22.57	6.9	<5.0	50	500	14
Mercury	1.29	0.31			0.3	0.1	0.08
Nickel	125.3	19.43	5.0	<2.0	30	200	11
Selenium	2.41	<0.54			1	70	0.3
Silver	3.30	<0.67			2	-	-
Vanadium	51.90	12.46	5.7	<4.0	2.5	-	43
Zinc	3853	103.27	18.2	<2.5	50	100	40
Nutrients (mg kg^{-1} DW)							
Nitrate-N	ND	ND	122.6	3.93			
Ammonia-N	ND	27.50	ND	ND			
Infinite-sink P	ND	6.94	14.03	0.6			
Total-K	ND	ND	ND	5.3			26000
Other							
pH $_{water}$	6.44	7.31	5.79	7.06			
OM (%DW)	14.00	1.94	76.29	1.33			
DW (%FW)	86.70	93.11	75.0	99.6			
BD (g DW/ mL)	0.70	1.85	1.27	1.12			

[1] Critical Stressor Concentration Levels (CSCL, USEPA, 1999) based on screening benchmarks are given for comparison, and levels in the Eastern U.S. are given for reference. Mean values (N=3).
[2] Benchmark Fluorene, 56-day exposure.
Abbreviation: ND, not determined.

Table 2 Characteristics of the Dredged Material Mixtures Prior to Incubation					
	Dredged Material Mixture				
Characteristic	Reference	10% IHC	20% IHC	33% IHC	70% IHC
Organics (mg kg^{-1} DW)					
A-1248	0	10	17	24	46
Σ16 PAHs	0.32	66.1	114.7	183.2	336.3
Total Metals (mg kg^{-1} DW)					
Arsenic	3.49	8.81	15.30	21.43	28.10
Cadmium	0.65	2.44	4.69	72.80	116.67
Chromium	16.93	79.13	150.00	236.33	334.67
Copper	19.77	49.20	82.90	115.33	165.67
Lead	22.57	109.03	230.33	316.67	494.67
Nickel	19.43	31.43	45.03	59.97	78.77
Vanadium	12.46	13.26	11.63	16.63	13.27
Zinc	103.27	530.00	1063.33	1503.33	2420.00
DTPA-extracted Metals (mg kg^{-1} DW)					
Arsenic	0.03	0.05	0.07	0.10	0.15
Cadmium	0.49	1.06	1.56	2.44	4.35
Chromium	0.06	0.01	0.02	0.04	0.03
Copper	7.22	12.93	18.13	26.17	40.90
Lead	4.23	20.03	33.97	53.83	17.80
Nickel	1.06	2.10	3.22	4.35	6.82
Zinc	14.33	109.50	228.33	522.67	640.00
Nutrients (mg kg^{-1} DW)					
NH_4-N	27.50	60.87	98.00	126.00	224.00
Infinite-sink-P	6.94	5.73	2.39	4.60	3.83
Other					
pH water	7.31	7.22	7.20	7.17	7.19
Organic Matter (%DW)	1.94	3.16	4.38	6.28	10.06
Organic Carbon (mg g^{-1}DW)	11.25	18.33	25.40	36.42	58.35
Dry Weight (%FW)	93.11	91.67	91.66	89.93	88.10
Bulk Density (gDW mL^{-1})	1.85	2.08	1.89	1.50	0.92
CEC (meq g^{-1} DW)	10.2	ND	ND	ND	12.2

[1] DTPA-extractable vanadium was below detection. Target levels of nutrients were established gradually after initiation of the experiment. Mean values (N=3).
Abbreviations: IHC, Indiana Harbor Canal; ND, not determined.

Table 3
Responses of *L. perenne* After 59-day Incubation[1]

Plant Characteristic	Reference		10% IHC		20% IHC		33% IHC	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Biomass								
Shoots (g DW m^{-2})	65.83±3.94	95.79±11.56A	19.21±4.50	57.89±6.28B	3.13±0.75	19.10±3.40C	0.59±0.20	3.98±1.42D
Roots (g DW m^{-2})	15.71±2.28	51.46±8.87	8.08±1.53	19.93±7.26	1.56±0.71	6.74±2.72	0	0
Total (g DW m^{-2})	81.54±3.88	147.25±13.00	27.30±5.22	77.83±3.64	4.69±1.00	25.84±4.39	0.59±0.20	3.98±1.42
Lipids shoots (% DW)	7.30±0.39	6.20±0.72	9.23±3.58	6.82±1.25	13.48±2.53	4.61±0.83	18.83	7.85
Lipids roots (% DW)	2.80±0.76	2.69±1.07	4.50±0.79	3.71±0.07	8.54	3.37±0.52		
Organics in Shoots (mg kg^{-1} DW)								
Arochlor-1248	0	0A	0.61±0.05	0A	0	0A	0	14.25B
Σ16-PAHs	0	0.20±0.16A	2.51±0.15	0.31±0.04A	6.36±5.57	1.73±1.29A	51.87	29.89B
Metals in Shoots (mg kg^{-1} DW)								
Arsenic	0.31±0.45	0.48±0.00	1.68±0.36	0.96±0.09	3.36±1.07	2.38±1.70	1.39±1.96	3.70±1.36
Cadmium	0.59±0.08	0.64±0.13	0.80±0.19	0.41±0.04	2.41±0.35	1.09±0.66	1.85±0.60	1.21±0.09
Chromium	21.55±10.54	7.38±2.57	22.75±6.01	10.11±2.53	35.10±7.07	35.25±11.10	19.55±7.00	31.05±9.26
Copper	13.20±0.99	14.10±0.71	23.85±0.49	24.20±2.97	47.70±4.67	31.10±0.61	42.75±9.40	64.70±5.37
Lead	4.74±1.92	3.81±1.46	20.90±13.01	8.51±1.25	49.70±20.36	33.45±31.04	27.05±8.41	51.90±21.07
Nickel	11.995±5.10	5.82±0.92	12.20±0.28	9.50±0.27	19.15±2.19	15.40±4.53	16.70±3.54	22.95±2.90
Zinc	82.10±19.09	81.55±11.10	197.00±33.94	231.5±30.41	315.50±74.25	278.00±118.79	270.50±84.15	ND
Organics in Roots (mg kg^{-1} DW)								
Arochlor-1248	0	0A	16.01±6.22	9.21±4.73AB	22.38±21.84	6.10±10.57B		
Σ16-PAHs	0	0.06±0.10A	6.05±1.54	7.14±4.65AB	25.69±11.28	6.40±5.82B		
Metals in Roots (mg kg^{-1} DW)								
Arsenic	2.04±0.14	2.40±0.33	2.16±0.52	3.08±0.11	3.53±1.07	3.18±0.42		
Cadmium	3.31±0.06	1.84±0.18	2.47±0.16	0.19±0.07	2.83±0.18	0.49±0.19		
Chromium	15.20±1.84	30.25±1.48	14.25±3.46	24.50±3.39	59.40±63.07	15.85±3.18		
Copper	47.55±5.73	56.65±1.91	73.05±1.63	75.00±2.69	71.80±8.91	68.90±3.96		
Lead	8.82±0.09	19.35±12.23	11.62±4.21	34.95±22.42	41.80±29.13	24.80±8.91		
Nickel	15.50±0.00	24.60±2.62	17.80±1.70	19.50±2.40	22.25±0.49	19.40±2.55		
Zinc	130.5±2.12	162.50±2.12	226.50±2.12	304.50±44.55	406.50±193.04	282.50±24.75		

[1] Mean values and standard deviations (biomass N=5, organics N=3; metals N=2). Different letters indicate statistically significant differences between means at the 95-percent confidence level, according to Fisher's least significant difference procedure.
Abbreviations: IHC, Indiana Harbor Canal; ND, not determined.

Table 4
Responses of *E. fetida* After 28-day Incubation[1]

Worm Characteristic	Dredged Material Mixture				
	Reference	10% IHC	20% IHC	33% IHC	70% IHC
Biomass (g DW m⁻²)					
Biomass (g DW cyl⁻¹)	1.56±0.07A	1.35±0.01AB	1.20±0.01BC	0.81±0.01C	0.05±0.01D
No individuals (N cyl⁻¹)	19.6±0.1A	19.4±0.8A	19.4±0.8A	17.6±0.8A	1.6±0.8B
Lipids (% DW)	10.26±1.49	9.71±0.34	10.78±1.65	12.12±2.82	32.19
Organics (mg kg⁻¹ DW)					
Arochlor-1248	0A	33.27±1.25B	45.26±4.94B	44.90±19.68B	212.19C
Total-PAHs	0.34±0.50A	38.55±3.38AB	63.47±9.75BC	85.30±39.48C	449.76D
Metals (mg kg⁻¹ DW)					
Arsenic	5.63±0.56	4.74±1.82	3.84±1.82	3.24±0.35	ND
Cadmium	7.28±1.76	3.78±2.97	5.68±4.21	1.67±1.44	ND
Chromium	1.66±0.07	4.39±1.40	3.01±2.41	2.84±2.46	ND
Copper	12.65±0.21	15.25±1.20	16.8±0.14	16.65±5.02	ND
Lead	1.11±0.43	6.59±1.39	4.74±3.59	5.33±3.73	ND
Nickel	2.61±0.57	3.55+1.33	3.53±0.44	2.79±0.42	ND
Zinc	82.4±3.0	113.5±3.5	108.0±5.7	108.7±23.1	ND

[1] Mean values and standard deviations (biomass N=5, organics N=3; metals N=2). Different letters indicate statistically significant differences between means at the 95 percent confidence level, according to Fisher's least significant difference procedure. Abbreviation: IHC, Indiana Harbor Canal; ND, not determined.

Table 5
Relationships Between Individual Organic Compounds in Plants and Worms,[1] in C18 Disks,[2] and in the DM Mixtures[3] (R^2 values of linear regressions between the log transforms of plant, worm, C18-disks, and those of the DM mixtures[4] are listed below)

Organic Compound	Abbr.	Log K_{ow}[5]	Vapor Pressure (Pa at 25°C)[5]	70% IHC DM-mixture (mg kg^{-1}DW)	R^2 Plant Shoot	R^2 Plant Root	R^2 Worm	R^2 Disk
Arochlor-1248	BZ22	5.58	0.5×10^{-3}	46.1	BD	ns	ns	0.69
Naphthalene	NAP	3.36	10.80	11.93	ns	ns	ns	ns
Acenaphtylene	ACY	4.07	3.87	2.73	BD	-	ns	0.81
Acenaphthene	ACE	3.92	3.07	12.57	-	-	0.76	0.63
Fluorene	FLU	4.18	1.13	10.37	0.65	ns	ns	0.61
Phenanthrene	PHE	4.46	0.018	44.70	0.34	ns	ns	0.65
Anthracene	ANT	4.54	0.001	10.60	ns	0.98	ns	0.62
Fluoranthene	FLA	5.22	0.001	47.17	0.42	ns	0.43	0.73
Pyrene	PYR	5.18	0.0006	42.80	0.63	0.28	ns	0.74
Benzo(a)anthracene	BAA	6.25	1.4×10^{-8}	22.07	0.70	ns	ns	0.77
Chrysene	CHR	5.86	5.7×10^{-7}	22.90	-	-	ns	0.72
Benzo(b)fluoranthene	BBF	6.57	5×10^{-7}	28.83	0.63	ns	0.40	0.80
Benzo(k)fluoranthene	BKF	6.40	2.07×10^{-5}	8.97	ns	ns	ns	0.76
Benzo(a)pyrene	BAP	5.91	0.28×10^{-3}	18.97	ns	ns	ns	0.78
Indeno(1,2,3c)pyrene	IND	6.71	1×10^{-10}	23.40	-	-	0.59	0.72
Benzo(ghi-)perylene)	BGHI	7.23	1.39×10^{-8}	22.67	-	-	0.44	0.77
Dibenzo(a,h)anthracene	DIB	6.75	3.70×10^{-10}	5.83	BD	BD	0.61	ns

[1] Relationships in plants and worms expressed as ^{10}log mg compound per kg lipid.
[2] Relationships in C18 disks expressed as ^{10}log g compound per kg disk and kg soil-DW.
[3] Relationships in the DM mixtures expressed as ^{10}log mg compound per kg organic carbon.
[4] Arochlor-1248 and PAH concentrations in the DM-mixture with the 70-percent contribution of IHC DM are given for comparison.
[5] Selected physico-chemical characteristics of the organic contaminants are given for reference (Hawker and Connell 1988; Mackay, Shiu, and Ma (2000).
Abbreviations: IHC = Indiana Harbor Canal; BD = below detection; ns = not significant (insufficient data for statistical analysis).

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